Mouse analog of micvotabart pelidotin sensitizes a refractory syngeneic breast cancer model to anti-PD1 therapy

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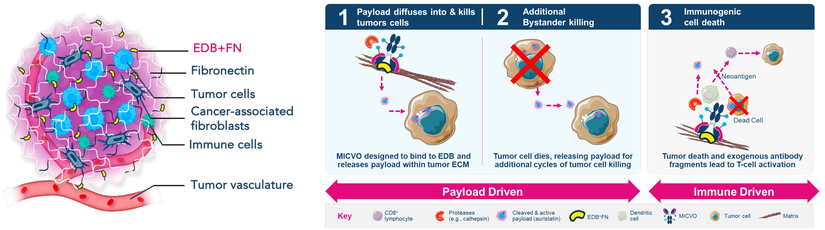
Abstract: A115

Background

- Antibody-drug conjugates (ADCs) are transforming cancer therapy, with some augmenting the efficacy of checkpoint immunotherapy [1,2].
- Micvotabart pelidotin (MICVO, aka PYX-201) is a first-in-concept ADC targeting extradomain-B of fibronectin (EDB+FN), a non-cellular structural component within the tumor extracellular matrix that is highly expressed in tumors compared to normal adult tissues [3].
- Preliminary results from a Phase 1, Part 1 clinical trial (**NCT05720117**) demonstrated that MICVO exhibited favorable anti-tumor activity across multiple solid tumor types.
- In preclinical models, a mouse analog of MICVO converted immune-excluded EMT6 triple negative breast cancer (TNBC) tumors into T-cell–infiltrated, inflamed tumors, thereby enhancing their responsiveness to anti–PD1 therapy [4].
- **The objective of this poster is to demonstrate that the mouse analog of MICVO modulates the tumor immune microenvironment and enhances anti–PD1 efficacy in the 4T1 TNBC syngeneic model otherwise refractory to immunotherapy**. In particular, a subset of T cells known as progenitor exhausted T cells (Tpex) were evaluated due to their critical role in response to immunotherapy [5].

Mechanism of action for micvotabart pelidotin (MICVO)



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Methods

- The mouse analog of MICVO (maMICVO) consists of L19-derived variable regions fused to a mouse IgG2a backbone and conjugated to an Auristatin0101 payload via an mcValCitPABC cleavable linker, with an average drug-to-antibody ratio of 4 [6].
- Eight-week-old immunocompetent female Balb/c mice were subcutaneously (S.C.) injected with 0.5 × 10^6 4T1 or 1.0 x 10^6 EMT6 TNBC cells into the right flank.
- Once tumors reached ~150 mm^3 (Day 0), mice were randomized and treated with maMICVO (Q4Dx3), anti-mouse PD1 (Clone RPM1-14; Q3Dx4), or the combination of both. Body weight and tumor volumes were monitored throughout the study.
- At selected timepoints, tumors were harvested and either enzymatically digested for flow cytometry or fixed and paraffin-embedded for immunohistochemistry (IHC).

maMICVO inhibits growth of EDB+FN-low 4T1 tumors

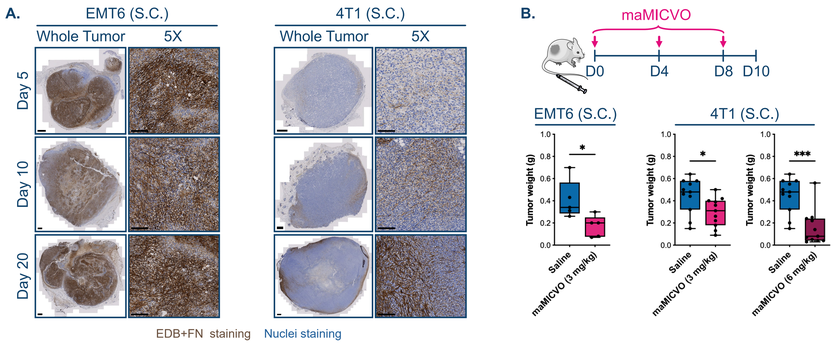
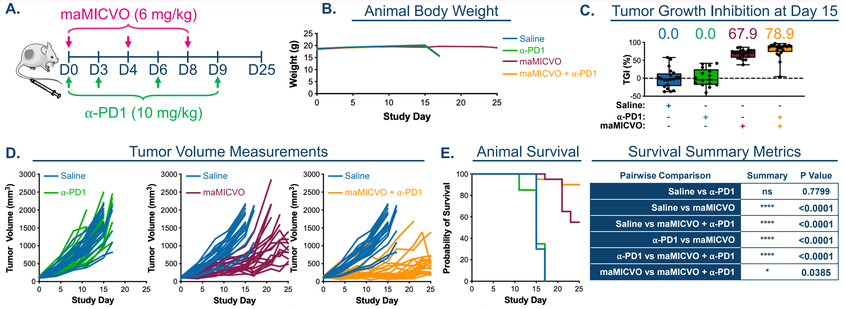


Figure 1. Mouse analog of MICVO inhibits growth of syngeneic 4T1 tumors despite their low expression of EDB+FN. **(A)** IHC analysis of untreated EMT6 and 4T1 tumors harvested at indicated timepoints post-inoculation showed that 4T1 tumors consistently expressed lower levels of EDB+FN compared to EMT6. **(B)** In efficacy studies, maMICVO treatment reduced EMT6 tumor growth and, notably, also inhibited 4T1 tumor growth in a dose-dependent manner, with 6 mg/kg producing the strongest effect despite the low expression of EDB+FN in the 4T1 model. P-values were determined by Mann–Whitney U test; ns, P > 0.05; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

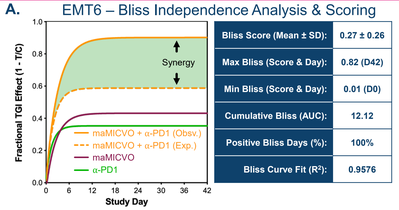
maMICVO sensitizes refractory 4T1 tumors to anti-PD1



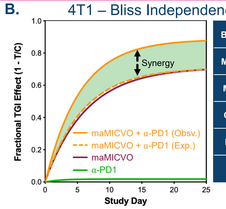
Pairwise Comparison	Summary	P Value
Saline vs α-PD1	ns	0.7799
Saline vs maMICVO	****	<0.0001
Saline vs maMICVO + α-PD1	****	<0.0001
α-PD1 vs maMICVO	****	<0.0001
α-PD1 vs maMICVO + α-PD1	****	<0.0001
maMICVO vs maMICVO + α-PD1	*	0.0385

Figure 2. Mouse analog of MICVO sensitizes immunotherapy-refractory 4T1 tumors to anti-PD1. **(A)** 4T1-bearing mice were treated with maMICVO, anti-PD1, or the combination. **(B)** All treatments were well tolerated. Body weight loss was observed with increased tumor burden in saline and anti-PD1 treatment groups. **(C)** As reported previously, anti-PD1 alone had no effect on tumor growth, whereas maMICVO alone inhibited growth, and the combination produced the strongest suppression. **(D–E)** Spider and survival plots confirm superior efficacy of the combination, with survival analyses demonstrating significant benefit over single agents. Together, these results demonstrate that maMICVO sensitizes 4T1 tumors to anti-PD1. Data combine 2 experiments with n=10 mice per group each. P-values were determined by Holm–Šídák test; ns, P > 0.05; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

maMICVO + anti-PD1 demonstrates synergistic anti-tumor activity



Bliss Score (Mean ± SD):	0.27 ± 0.26
Max Bliss (Score & Day):	0.82 (D42)
Min Bliss (Score & Day):	0.01 (D0)
Cumulative Bliss (AUC):	12.12
Positive Bliss Days (%):	100%
Bliss Curve Fit (R^2):	0.9576



Bliss Score (Mean ± SD):	0.15 ± 0.07
Max Bliss (Score & Day):	0.23 (D8)
Min Bliss (Score & Day):	0.00 (D0)
Cumulative Bliss (AUC):	3.72
Positive Bliss Days (%):	89%
Bliss Curve Fit (R^2):	0.9985

Figure 3. Mouse analog of MICVO synergizes with anti-PD1 for improved anti-tumor activity. **(A–B)** Bliss independence modeling was applied to assess synergy between maMICVO and anti-PD1 using tumor volume data from previously reported EMT6 combination studies [4] and the 4T1 model described in Figure 2. Fractional tumor growth inhibition (1 − T/Control) defined single-agent effects, the Bliss-expected outcome (eA + eB − eA × eB) represented the predicted additive effect, and the combined response expected if maMICVO and anti-PD1 acted independently (effects modeled with nonlinear regression). In both models, the observed combination of maMICVO and anti-PD1 exceeded the activity of either monotherapy and the predicted additive effect. The shaded green region represents the delta between observed and expected outcomes, illustrating the magnitude of synergy. This effect was strongest in EMT6 tumors; however, the 4T1 model also showed a synergistic benefit. Together, these findings demonstrate that maMICVO and anti-PD1 act synergistically across models, with maMICVO appearing to modulate tumors to enhance responsiveness to checkpoint blockade.

maMICVO drives the influx of Tpex into 4T1 tumors

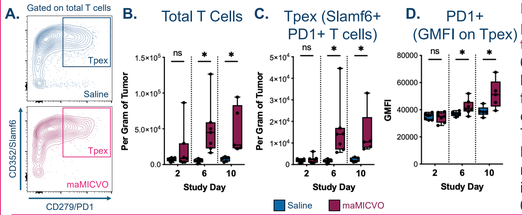


Figure 4. maMICVO drives infiltration of progenitor exhausted T cells (Tpex) into 4T1 tumors. **(A)** 4T1-bearing mice were treated with 6 mg/kg maMICVO (Q4Dx3), and tumors were harvested 48 hr after each dose (D2, D6, D10) for evaluation of immune infiltration by flow cytometry. **(B)** maMICVO increased intratumoral T cells, including **(C-D)** Tpex with higher surface PD-1 expression. These findings suggest that maMICVO enriches a PD-1–sensitive T cell pool in 4T1 tumors. Mann–Whitney U test; ns, P > 0.05; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

Conclusions

- Monotherapy with maMICVO suppressed 4T1 tumor growth, demonstrating potent anti-tumor activity despite low EDB+FN expression.
- Combining maMICVO with anti-PD1 further improved outcomes, with Bliss independence analysis revealing synergistic activity. In particular, these data suggest maMICVO converted immunotherapy-refractory 4T1 TNBC tumors into anti-PD1 responsive tumors.
- Treatment with maMICVO was also associated with increased intratumoral T cells, particularly progenitor exhausted T cells, a subset highly responsive to PD-1 blockade.
- Additional studies are ongoing to further explore maMICVO-induced immune changes in vivo.
- Together, these findings support the clinical development of MICVO in combination with pembrolizumab for the treatment of difficult-to-cure cancers (NCT06795412).

References

[1] Hoimes et al., Future Oncology 2024 March; 20(7):351-360.
[2] Powles et al., N Engl J Med 2024 Mar; 390(10):875-888.
[3] Lewandowski et al., Cancer Res 2024 March; 84(6_Supplement):2908.
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[6] Hooper et al., Mol Cancer Ther 2022 Sep;21(9):1462-1472.

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